REMARKS

Reconsideration and withdrawal of the rejections of the application are requested in view of the amendments and remarks presented herein, which place the application into condition for allowance. The Examiner is thanked for indicating that the Sequence Listing, drawings, priority documents and Information Disclosure Statement have been received, considered, and appear to be in good order. The Examiner and Primary Examiner are also thanked for courtesies extended during the interview on January 13, 2004, during which the herein-presented amendments and arguments were discussed.

I. STATUS OF CLAIMS AND FORMAL MATTERS

Claims 1-32 and 34-41 are pending in this application; claims 1-21, 28 and 34-41 are under consideration. It is requested that claims 22-32 be rejoined and examined with claims 1-21, 28 and 34-41, for reasons discussed below. Claims 1-28 are amended, claim 33 is cancelled and claims 34-41 are added. Support for the amended claims can be found throughout the specification, and from the claims as originally filed. Particular support for claims 34 and 35 can be found, for example, on page 7, line 26. Particular support for claim 41 can be found in Table 4. The CDR-1 loop is increased by one amino acid in clones 3M-3, 3M-6, 3M-7, 3M-11, 3N-13 and 3N-14. The CDR-3 loop is increased by one amino acid in clone 3M-20. The majority of the amendments place the claims in better condition, and do not affect scope. No new matter is added.

It is submitted that the claims, herewith and as originally presented, are patentably distinct over the prior art cited by the Examiner, and that these claims are and were in full compliance with the requirements of 35 U.S.C. §112. The amendments of and additions to the claims, as presented herein, are not made for purposes of patentability within the meaning of 35 U.S.C. §§§§ 101, 102, 103 or 112. Rather, these amendments and additions are made simply for clarification and to round out the scope of protection to which Applicants are entitled. Furthermore, it is explicitly stated that the herewith amendments should not give rise to any estoppel, as the herewith amendments are not narrowing amendments.

Specification

A new Abstract is submitted herewith, removing the word "novel", and thereby overcoming the objection.

Claim Objections

Claim 19 has been amended to correct the Markush format, thereby overcoming the objection. Claim 33 has been cancelled, rendering its objection moot.

II. THE REJECTIONS UNDER 35 U.S.C. §112, 2ND PARAGRAPH, ARE OVERCOME

Claims 1-10, 13-21, 28 and 33 were rejected under 35 U.S.C. §112, second paragraph, as allegedly being indefinite. The rejection is traversed.

The Office Action alleges that the recitation of "V-like domain (VLD) derived from a non-antibody ligand" is indefinite because the metes and bounds of the limitation are unclear. As discussed in MPEP §2173.02:

> Definiteness of claim language must be analyzed, not in a vacuum, but in light of:
> (A) The content of the particular application disclosure;
> (B) The teachings of the prior art; and
> (C) The claim interpretation that would be given by one possessing the ordinary level of skill in the pertinent art at the time the invention was made.

As regards the disclosures in the specification itself, the Examiner acknowledges (page 3 of the Office Action), that a "V-like domain" is defined on page 5 of the specification as one which has "*similar structural features to the variable heavy or variable light domains of an antibody*". The next paragraph of the specification (spanning pages 5 and 6) provides examples of suitable non-antibody ligands which may provide V-like domains suitable for the invention. Table 1 on page 6 then lists these molecules and makes specific reference on page 6, lines 16-18, to the disclosure in The Leucocyte Facts Book (1993) Eds. Barclay *et al.*, Academic Press, London ("Barclay *et al.*"). The entire text of Barclay *et al.* is incorporated by reference into the present specification. The specification must therefore be read in conjunction with the disclosures in Barclay *et al.*, and the content of Barclay *et al.* must be taken into consideration when analyzing the definiteness of claim language.

Barclay *et al.* provides a detailed analysis of different protein superfamilies and the nature of the domains associated with each superfamily. Chapter 1 of Barclay *et al.* (attached as part of Appendix J) introduces, in broad general terms, the basic molecular and structural organization, including different types of domains, associated with leucocyte surface antigens. Figure 1 on page 3 of Barclay *et al.* depicts models for domains and repeats found in the membrane proteins. "Ig V set", "Ig C1 set", Ig C2 set" and "Fibronectin type III" are shown as four distinct types of domains.

Table 1, spanning pages 4-8 of Barclay *et al.*, details the distribution of domains in leucocyte surface molecules. A number of domain types fall under the "umbrella" of extracellular domains, including FN type IIISF (ie. Fibronectin type II Superfamily) and IgSF (ie. IG Superfamily) domains.

As stated on page 2, final paragraph, of Barclay *et al.*, the criteria for defining the superfamily relationships of protein domains are discussed in Chapter 3 (attached as Appendix I). The introduction section of Chapter 3, on page 38, introduces the concept of conserved residues within domains of members of the immunoglobulin superfamily (IgSF) as relating to important structural features of the domain. In the final paragraph on page 38, the term "domain" is defined as relating to a segment of sequence that forms a discrete structural unit. The "variable heavy or variable light antibody" domains referred to in the definition on page 5 of the present specification are Ig domains. The Barclay *et al* reference makes it clear that these domains are structurally distinct from other domains such as fibronectin (FN) type III domains.

Pages 39 through 46 of Barclay *et al* discuss the various methods for allocating protein domains to particular families. The material on page 45 makes it clear that a combination of structural similarity and sequence similarity as defined through computer analysis is required to define a domain type. The paragraph starting at the bottom of page 44 explains that various different sequence types can give rise to Ig fold structures but when these are also analyzed for conserved sequence features, it becomes clear that they fall into different families.

An informative discussion of the IgSF begins on page 54 and continues on page 59, reading, in part:

> "The structures of several IgSF domains have been determined by X-ray crystallography including Ig V- and C-domains..... These structures show that the IgSF domains characterized by sequence similarities over about 100 amino acids correspond to structural units with distinct folding patterns referred to as the Ig-fold... The Ig-fold consists of a sandwich of two β- sheets, each consisting of antiparallel β- strands of 5-10 amino acids with a conserved disulphide between the two sheets in most but not all domains. The sequence similarities are mainly found at the positions of in-pointing residues in the β- strands with considerable differences in the loops that connect the strands and the out-pointing residues on the faces of the β- sheets. The core of the fold is made up of three β- strands labeled ABE and GFE and the positioning of these is shown in the various folds illustrated in Fig. 9. **The folds vary considerably in length in the middle of the sequence with Ig V-domain folds being the archetype for the longer fold. The extra sequence in comparison with C-domains forms an additional pair of β strands (C' and C" in Fig. 9) and the connection between these forms the**

second complementarity determining region in antibody and TcR V-domains"

Figure 9, on page 56, shows the folding pattern of IgSF domains, including two examples of "Ig V" set domains: V_H of human NEW Fab and domain 1 of rat CD2, both of which have 9 β strands. In comparison, β-2 microglobulin and domain 2 of human CD4 are described as "Ig C1" and "Ig C2" sets, respectively, and both have 7 β strands. Figure 10, on pages 56 and 57, depicts sequence alignments of Ig V-set domains from various proteins. Included in these are CD2, CD4 and CD8, all of which fall within the scope of the present invention and are listed in Table 1 on page 6 of the specification.

In conclusion, based on the definition for V-like domain provided on 5 (lines 25-27) of the present specification, and reading this is conjunction with the disclosures in Barclay *et al.*, the term "V-like domain" would be perfectly clear to a skilled addresse. Domain is a term of the art as is clear from the discussion in Barclay *et al.*, and, V-like domains are domains having similar structural features to the variable heavy or variable light domains of an antibody, as explicitly stated in page 5, lines 25-27, of the present specification. Barclay *et al.* clearly discloses and describes in detail V-like domains, although these are referred to as "Ig V-set domains".

Similar features that the V-like domains of the present invention have in common with the variable heavy or light domains of antibodies are CDR loop structures, as stated on page 5, lines 27-30, of the specification.

Another characteristic of the V-like domains that are derived from non-antibody ligands, as recited in claim 1, is that they do not associate with one another to make Fv-type molecules, as explained in the paragraph beginning on page 2, line 17, of the specification. Therefore, the application discloses structural features of V-like domains, consistent with requirement (A), above.

Although we submit that the term "V-like domain" is clearly and definitively described in the specification *per se*, the teachings of the prior art further support this assertion. The prior art teachings are entirely consistent with the definition of V-like domain provided by the specification. It should be noted that the phrases "V-like extracellular domain" and "V-like domain" are used extensively with reference to CD28 and CTLA-4 in the Peach *et al.* reference, cited by the Examiner. Also, enclosed are three publications demonstrating that the terms "V-

like domain" or "V-like region" were standard in the art. For example, beginning at column 6, line 6, of U.S. Patent No. 5,336,603 (Appendix A) the "V-like regions" of CD4 are described in detail. Likewise, column 2, lines 12-16, of U.S. Patent No. 5,484,892 (Appendix B) mention the V-like domain of CD22. Wilson *et al.* (Appendix C) cloned the cDNA of CD22, and were able to compare the structure of its V-like domain to V-like domains of carcinoembryonic-associated antigen (CEA), myelin-associated glycoprotein (MAG) and B lymphocyte-cell adhesion molecule (BL-CAM). Note that Wilson *et al.* was published in January 1991, more than seven years before the earliest priority date of the instant application. These references provide evidence of the acceptance and common knowledge of the term "V-like domain" in the prior art before the date the current invention was made. Furthermore, they provide examples of several molecules, other than CTLA-4, that contain V-like domains and could be used in the instant invention.

There are several on-line resources that are dedicated to providing structural information with respect to sequences and structures of immunoglobulins and immunoglobulin-like molecules. Although it cannot definitively be demonstrated what information was available electronically at the time the present application was filed, the currently available information provides some insight into the claim interpretation that one of ordinary skill in the art would have given at the time the invention was made. For example, Giudicelli *et al.* (Appendix D) was published in 1997, prior to the earliest priority date of the instant application. It details the establishment of an integrated database, the International ImMunoGeneTics (IMGT) database "to establish rules for describing immunoglobulin and TcR [T-cell Receptor] sequences of any species" (page 206). This database also includes related proteins of the immune system, such as cell surface proteins. Definitions and functional characteristics of the various regions of these molecules are provided in the database, which was first publicly available in July, 1995. (See page 10 of Lefranc *et al.*, 2003; Appendix E.) A copy of the current feature definitions is attached (Appendix F) to provide an example of the type of information that is available through the IMGT database. Although this does not directly demonstrate the state of the art at the time the application was filed, since the term "V-like domain" was common in the at the time of filing, and since the IMGT database was publicly available at the time of filing, it is reasonable to conclude that the skilled artisan would have understood what a V-like domain was, and what the current claims encompass.

It is clear, from the disclosure in the specification and from the state of the art at the time the application was filed, that proteins having a V-like domain are not characterized by "some undefined criteria", as alleged in the Office Action, but rather, have the characteristics of a V domain, one of which is complementarity determining region (CDR) loop structures. The publication by Bork *et al.* (Appendix G), published in 1994 and discussed in greater detail below, clearly separates V-like domains (VLDs) from other domains falling within the immunoglobulin superfamily (IgSF) by the presence of nine β-sheets in the protein structure. These β-sheets provide for the existence of intervening loop regions at the face of the molecule, which is remote from the cell surface attachment site. These loop regions are equivalent to the CDR regions of antibody V-regions and are discussed herein as CDR loop structures. As defined in the art, molecules having a V domain are necessarily immunoglobulins (Ig) or T-cell receptors (TcR). In a molecule that is not an Ig or a TcR, the region sharing the same structural features as the V domain of an Ig or a TcR is referred to as a V-like domain. Further details regarding the characteristics of V-like domains are provided below. It is submitted that, when analyzed in view of MPEP §2173.02, the claims meet the requirements of 35 U.S.C. §112, second paragraph.

Claim 21 was rejected under 35 U.S.C. §112, second paragraph, as lacking antecedent basis for "multivalent reagent". As suggested by the Examiner, claim 21 has been amended to depend from claim 1 or claim 20, obviating the rejection.

In view of the foregoing, reconsideration and withdrawal of the rejections under 35 U.S.C. §112, second paragraph, are requested.

III. THE REJECTIONS UNDER 35 U.S.C. §112, 1ST PARAGRAPH, ARE OVERCOME

Claims 1-10, 13-21, 28 and 33 were rejected under 35 U.S.C. §112, first paragraph, as allegedly lacking adequate written description and enablement. The rejections are traversed.

The basis of these rejections appears to be based in a misunderstanding of the claimed invention, particularly with respect to the VLD. As discussed above, the metes and bounds of what constitutes a VLD are not ambiguous, particularly in light of the disclosure in Barclay *et al.* The attached review article by Bork *et al.* (Appendix G), published in 1994, relates generally to the characteristics of Ig-like domains (ILDs), and further helps to clarify the structural and functional aspects of VLDs. As explained by Bork *et al.* in the Introduction, molecules with Ig-like domains, such as VLDs, can have divergent sequences from Ig molecules, but can have the same secondary structure and topological characteristics. Therefore, the definition of a VLD

does contain the inherent negative limitation that it not be derived from an antibody or TcR, but that fact does not equate to an uncertain description of what the genus does encompass. On page 310, Bork *et al.* state that they wish to address the very issues that the Examiner raises in the Office Action, *e.g.* whether there are conserved features common to all ILDs, and more specifically to this application, to all VLDs. The study includes careful analysis of several different Ig and non-Ig molecules from various species (as listed in Table 1), and concludes there are indeed conserved features, which are divided into four different subtypes. (See the first full paragraph on page 312 and Figures 1 and 2.) As discussed on page 312 and shown in Figure 1, the V-type domain contains a nine-stranded β-sheet topology, as occurs in the V domain of Ig molecules. Further, Figure 2 demonstrates that statistical analysis of the structural relationships of different Ig domains clearly differentiates a class of V- and V-like domains from other classes, such as S, C and H. Therefore, by 1991, an approach already existed to determine whether a molecule contained a VLD or not.

The teachings provided by Bork *et al.* in 1994 have been accepted in the art, and a synopsis of those findings can be found on the website of the Jenner Institute for Vaccine Research, where the IgSF is described (Appendix H). This tutorial describes the structure of a V region of Ig molecules, in detail, as being "dominated by a series of nine antiparallel β strands, connected by variable-length loop sequences, that assume a characteristic barrel or sandwich like structure with two β sheets, stabilized by the disulphide bridge. There are four beta strands in one sheet and three on the other. The extra pair of β strands is essentially situated between the faces of the sandwich." (Page 2.)

The instant specification refers to a V-like domain as being "similar" to an antibody heavy or light chain variable region because their secondary structures share common features, including the CDR loops and 9-stranded β-sheets. As stated in the Jenner Institute materials, "[a]s new members of the IgGSF have been characterized, their homology units have generally been defined as either V- or C-like, based on primary sequence similarities and secondary structure predictions. (*Id.*) Bajorath, cited by the Examiner, discusses consensus residues that shared by the VLDs of several molecules. (See the Abstract.) Therefore, there are predictable structural motifs and consensus sequences that are common to the genus of VLDs, and these are and were appreciated by the skilled artisan at the time of filing.

The disclosures in Barclay *et al.* are entirely in accordance with the above-referenced disclosures. Furthermore, the entire text of Barclay *et al.* is incorporated by reference into the present application, and therefore constitutes part of the disclosure of the present specification. Chapter 3 of Barclay *et al.*, and thus the present specification, provides detailed analysis of different protein superfamilies and the nature of the domains associated with each superfamily. The Examiner is referred to the discussion on pages 10 and 11 of this response.

The first paragraph on page 38 introduces the idea of conserved residues within a domain or superfamily relating to important structural features of the domain. At the bottom of the page 38, the term "domain" is also defined.

On pages 39 to 46, there is a discussion of various methods for allocating protein domains to particular families. The disclosures on pages 44 and 46 make it clear that a combination of structural similarity and sequence similarity as defined through computer analysis is necessary to define a domain type.

Barclay *et al.* therefore provides a clear disclosure of what constitutes a V-like domain (referred to in Barclay *et al.* as Ig V-set domains) and how molecules with such domains may be identified. As has been already been discussed, page 5, lines 25-27, of the specification states that "V-like domain" "is intended to refer to a domain which has similar structural features to the variable heavy (V_H) or variable light (V_L) antibody." This definition is consistent with what was known in the art with respect to VLDs. The Examiner is respectfully reminded that a specification need not disclose--and best omits--that which is well known in the art. *In re Buchner*, 929 F2d 660, 661, 18 USPQ2d 1331, 1332 (Fed. Cir. 1991). Therefore, a protracted discussion of VLD structure need not necessarily be present in the specification, as VLDs were already known and characterized in the art. Further, as Barclay *et al.* was incorporated by reference into the instant application, the disclosure found therein is considered to be part of the application.

In addition to their common structural features, VLDs also share an important functional property. In contrast to the V domains of Igs and TcRs, they have no propensity to form heterodimers, *i.e.* Fv-type molecules (see page 2, lines 16-20, of the specification). Rather, the V-like domains of the present invention are naturally monomeric or form homodimers (see page 2, lines 26-35, page 3, lines 14-19, and page 13, lines 5-7, of the specification).

Therefore, VLDs are clearly defined in the specification, in the claims, and in the art, both structurally and functionally. At this point, a brief review of the claimed invention may be useful as a basis for the remaining discussion. As explained above and in the Background of the Invention, the invention involves a particular class of non-antibody ligand molecules. These ligands bind to specific binding partners, and have domains, for example, V-like domains and/or C-like domains, which are structurally analogous to the V domains and C domains of antibodies and T-cell Receptors, but are referred to as being "V-like" or "C-like" because these ligands are not antibodies or TcR. This class of molecules is not ill defined or ambiguous, nor does it consist of proteins with "wished for criteria". Rather, it is a well-studied and well-characterized group of molecules that includes certain cell surface antigens and adhesion molecules. In this application, these molecules are called "non-antibody ligands". (See page 5, lines 31-33 and Table 1.) While not all non-antibody ligands comprise a VLD, only those that do comprise a VLD are encompassed by the pending claims. The binding specificity of the claimed non-antibody ligands lies in their VLDs, as is the analogous case for Ig molecules and their V domains. In order to further demonstrate that many proteins comprising VLDs were known and appreciated at the time the application was filed, the Introduction (pages 2-12) and selected pages of Section II of Barclay *et al.* is attached as Appendix J. These selections provide structural details of individual leukocyte surface antigens that are listed on pages 5 and 6 of the application.

Thus, the aim and benefit of the instant invention is at least two fold: (1) the binding specificities of monomeric V-like domain binding moieties can be changed by altering the size or disulfide bonding characteristics of at least one CDR loop structure or part thereof, and (2) the solubility of monomeric V-like domain binding moieties can be improved by modifying or replacing at least one CDR loop structure or part thereof. This solves two separate problems, the first of which is providing a basic framework for engineering novel binding moieties. As discussed above, the binding specificity of ligands containing a V-like domain is conferred by structural features of the domain, and specifically by the sequence and topology of the CDR loops. The specification provides an example of such a non-antibody ligand, CTLA-4, and provides several examples of the claimed VLDs. Example 3 of the specification teaches how to replace the CDR-1 or CDR-3 loop of CTLA-4 with the somatostatin polypeptide, and how to replace the CDR-2 loop of CTLA-4 with the haemagglutinin epitope. Example 4 teaches how to

replace the three CDR loops of CTLA-4 with the three CDR loops of an anti-lysozyme antibody, cAb-Lys3. Example 5 teaches how to replace the three CDR loops of CTLA-4 with the three CDR loops of an anti-melanoma antibody, V86. All of these engineered VLD binding moieties were more soluble than their unmodified counterparts, and displayed binding specificity with respect to the newly added sequence that replaced the loop structure(s). Additional examples in the specification teach how to make and screen an entire library for novel VLD binding moieties.

The second problem solved by the instant invention is that of solubility. As discussed in the specification, production of only the extracellular domain, *i.e.* the VLD, of non-antibody ligands, particularly in bacterial systems, has been problematic due to a high degree of aggregation and a low level of production. The present inventors have produced a number of binding moieties with modified CDR loop structures in the VLD which have improved solubility compared with their unmodified counterparts. One of skill in the art can easily assess solubility using routine high performance liquid chromatography (HPLC) techniques.

The Office Action alleges, on page 4, that "the claims are drawn to a genus of 'binding moieties' structures which comprise *any* protein domain which can be considered by ambiguous criteria to be a "V-like domain". This statement reflects a misinterpretation of the invention. The claims are drawn to a genus of VLD binding moieties, and several members of this genus are described in the Examples of the specification. The backbone or scaffold of these binding moieties is derived from a non-antibody ligand comprising a VLD. As is discussed exhaustively above, this group of proteins is not ambiguous and is well known to artisans of ordinary skill. Moreover, it is not this VLD backbone or scaffold as a whole, but the modified region of the VLD, *i.e.* one or more CDR loops, that forms the "business" part of the claimed monomeric VLD binding moieties. It is the modification of this region that confers different binding and/or solubility properties, in comparison to the unmodified counterpart. Therefore, the example of CTLA-4 as the backbone or scaffold is representative of the genus of non-antibody ligands, because all that is required from this component of the invention is a VLD that can be modified. There is no reason to believe, nor has the Examiner provided any evidence demonstrating that the CDR loops of any VDL could not be similarly modified.

The Office Action alleges, on page 5, that a clear correlation between the structural aspects of the CTLA-4 VLD and its function as a "binding moiety" is lacking. As discussed above, it is the preservation of the CDR loop structures, supported by the presence of nine β-

sheets, that specifies the form of the structure, and defines VLDs. Thus, changes to CTLA-4 or other VLDs that preserve function as a "binding moiety" are necessarily those that preserve the overall nine β-sheet structure of the molecule. The bounds of these sequence requirements are discussed in Bork *et al.* Therefore, it is the modifications to the CDR loop structures of the VLD that correlate to its functional properties of modified binding and/or increased solubility. Accordingly, the claims do not need to be "limited to binding moieties which share a particular 'V-like domain'", as is suggested on page 6 of the Office Action. The choice of a combination of a suitable native CDR loop structure and a desired replacement sequence are well within the abilities of the skilled artisan, based on the teachings of the specification and the vast amount of structural data that was available on various members of the IgSF, specifically the V-like subset of those members, at the time the application was filed. And, although there is "variation in details of the structures" of VLDs, as is pointed out on page 6 of the Office Action, this variation does not make its function as a scaffold unpredictable. The Examiner cites Metzler *et al.* and Bajorath as evidence of the variability between VLDs, and suggests that undue experimentation would be required to "screen V domains from any of the large number of members of the IgSF which have V domains or 'V-like domains' at random and hope that another besides CTLA-4 could be identified". Both Metzler *et al.* and Bajorath state that CTLA-4 and ICOS, respectively, contain V-like domains. For example, page 527 of Metzler *et al.* states that "[t]he structure of CTLA-4ex is most similar to immunoglobulin superfamily variable-domains", and goes on to describe the β sheets. In addition, while the portion of Bajorath relied on by the Examiner discusses specific variations between CTLA-4 and ICOS, it should be noted that several similarities between ICOS and other family members are disclosed. For example, the Abstract of Bajorath states, "[d]espite low sequence identity, ICOS shares consensus residues characteristic of immunoglobulin variable-type domains with CD152 and CD28". The Examiner is reminded that there will be differences in areas responsible for binding specific molecules, but that these molecules all adopt similar Ig V-like folds comprising nine β-sheets. Therefore, there is no reason to expect that the skilled person could not reasonably predict that a V-like domain derived from ICOS would be suitable for use in the present invention.

The Examiner is also referred to the discussion in Chapter 3, pages 38 to 46, of Barclay *et al.* In the first paragraph on page 38 is states that in protein superfamilies, there is often only 15-25% sequence identity and at this level it can be difficult to be confident that a sequence match

indicates an evolutionary relationship, rather than just a chance similarity. An extensive discussion of how domains can be identified and categorized then follows.

Figure 10, on page 57 of Barclay *et al.*, shows the sequence alignment of ten Ig V-set domains for ten molecules. The positions of the 9 β strands (A,B,C,C',C",D,E,F,G) are indicated above the sequences. This demonstrates that it is well within the capabilities of the skilled addresse to use routine analytical tools to find molecules with V-like domains. Furthermore, it will be patently clear to the skilled addresse where the CDR loop structures are with the V-like domains. For example, the "CDR 2" loop structure connects the extra pair of β strands (C' and C") that are present in V-like domains, which are not present in C-like or FN type III domains (see page 59, last sentence of Barclay *et al.*). Similar sequence alignments in Metzler *et al.* (page 529) and Bajorath (page 171) show the 9 β strands and loop structures. The skilled addresse therefore has no difficulty in finding V-like domains and in knowing which parts of the V-like domains (ie, the CDR loop structures) should be modified or replaced in order to achieve the benefit(s) of the present invention.

In view of the foregoing, Applicants respectfully submit that adequate written description and enablement exist for the pending claims. The class of molecules known as "non-antibody ligands", belonging to the IgSF is well known and characterized, and the VLD of those non-antibody ligands that contain a VLD can be used as "scaffolds" in the claimed invention. It is not the VLD, *per se*, but the modification to the CDR loop structure(s) of the VLD that result in the claimed functions (improved solubility in claim 1, modified binding affinity in claims 7 and 8, and modified binding specificity in claim 9). Several working examples of CDR loop modifications are given in the application, providing a number of species to support the claimed genus of monomeric VLD binding moieties. Therefore, the Applicants were clearly in possession of the claimed invention at the time the application was filed.

Furthermore, it would present no undue burden to the skilled artisan to choose any member of the IgSF comprising a VLD and modify one or more of its CDR loops to improve solubility or modify its binding properties. The techniques for such modifications are routine molecular biology and are taught in the Examples, and the assays to determine whether solubility and/or binding have been affected as desired are routine biochemistry, for example, HPLC and antibody affinity assays. Determining whether undue experimentation is required to practice a claimed invention turns on weighing many factors summarized in *In re Wands* 8 U.S.P.Q.2d

1400 (Fed. Cir. 1988), including, (1) the quantity of experimentation necessary; (2) the amount of direction or guidance presented; (3) the presence or absence of working examples of the invention; (4) the nature of the invention; (5) the state of the prior art; (6) the relative skill of those in the art; (7) the predictability or unpredictability of the art; and (8) the breadth of the claims. While the claims are broad, practicing the invention would require only routine experimentation on the part of the skilled artisan. The amount of guidance provided in the application is high, particularly in view of the number of working examples presented. The state of the prior art is well developed in the sense that the molecules that are used as a basis for the invention were well known and described before the filing date of the present application. The section beginning on page 39 of Barclay *et al.*, (Appendix I) even provides details regarding how to identify superfamily domains of instance, for example, VLDs. The relative skill of those in the art is quite high, and a high level of unpredictability would not be expected. Therefore, enablement has been shown to exist.

Claim 28 was rejected under 35 U.S.C. §112, first paragraph, as allegedly lacking enablement. The Office Action states that claiming a "pharmaceutical composition" requires that the composition have an *in vivo* function. Claim 28 has been amended such that it no longer recites a "pharmaceutical composition", obviating the rejection.

In view of the amendments and arguments presented herein, reconsideration and withdrawal of the rejections under 35 U.S.C. §112, first paragraph, are requested.

IV. THE REJECTIONS UNDER 35 U.S.C. §102 ARE OVERCOME

Claims 1, 7, 10, 11, 13, 20, 21, 28 and 33 were rejected under 35 U.S.C. §102(b) as allegedly being anticipated by Peach *et al.* The rejection is traversed.

The claims have been amended so as to be directed to at least one modified monomeric non-antibody ligand V-like domain which is a binding a moiety, the solubility of the modified VLD being improved relative to the unmodified VLD. In view of the fact that Peach *et al.* only relates to CTLA4 molecules which are fused to an immunoglobulin fragment, it is submitted that claims as amended are novel over Peach *et al.*

The Office Action argues that, although Peach *et al.* is silent on the effect of the changes in the CDR loop structures on solubility, it shows, in Figure 4, that the chimeric proteins HS10, HS11, HS12 and HS13 each exist in monomeric form at a greater frequency than do either CD28 or CTLA4. The Office Action asserts that Figure 4 provides objective evidence that at least the

chimeric proteins HS4, HS4A, HS7, HS8, HS10, HS11, HS12 and HS13 have improved solubility, when compared with the unmodified VLDs of CD28 and CTLA4. This interpretation of Figure 4 of Peach is based on the erroneous premise that appearance of monomers in the gel samples is related to solubility. Solubility, as defined on page 7, lines 1-8 of the present specification, relates to lack of *aggregation* of monomers, as assessed by HPLC chromatography. Figure 4 of Peach does not measure aggregation as the samples are treated with SDS and then run on SDS gels. (See page 2051, col. 1, lines 8-12.) As any person skilled in the art will appreciate, SDS is a powerful ionic detergent that disrupts all protein-protein interactions. Therefore, protein A binding to Ig would be disrupted as a result of the SDS treatment, as would all intermolecular aggregation. Electrophoresis was performed in the presence of SDS, hence re-aggregation could not occur. So, the relative amount of monomers/dimers in the samples shown in Figure 4 of Peach *et al.* is not related to aggregation or solubility; rather, it is related to the presence (in dimers) or absence (in monomers) of disulfide bonds. Although SDS treatment disrupts protein-protein interactions, it does not disrupt covalent bonds. Since the mutant chimeric protein samples shown in Figure 4 were run under non-reducing conditions, disulfide bonds were preserved, and monomer and dimer forms of the CTLA-4 Ig proteins were observed from certain mutants. In contrast, the CD28 Ig and CTLA-4 standards were run under reducing conditions (which breaks disulfide bonds), and only one band (representing the monomer) was observed. (See page 2052, col. 2, lines 6-9.)

The Examiner has pointed out that monomers appear for some mutants in Figure 4, but not for others. As discussed by Peach *et al.*, the natural form of CTLA-4 and CD28 is a disulfide-linked homodimer. All of the fusion proteins analyzed in Figure 4 preserve the cysteine residue that is normally involved in homodimer formation (*e.g.* Cys 123 in CTLA-4). Whether or not monomers occur is most likely dependent on the accessibility of this cysteine residue in the different hybrid molecules, and the consequent efficiency of homodimer formation.

It appears as though the Examiner has assumed that the monomers of the chimeric proteins are more soluble than the dimeric forms; however, this is untrue. Rather, the monomer is an aberrant form of the molecule, whose natural form is a homodimer. There is nothing in Peach *et al.* to suggest that monomers are more soluble than dimers. Importantly, Peach *et al.* comment that both the parent molecules and chimeric proteins exist as oligomers, or aggregates,

and there is no suggestion that any of the mutant chimeric proteins behave differently with respect to aggregation. In particular, with regard to the parent molecules, Peach states, on page 2056, that "[d]espite repeated attempts, we have been unable to express soluble monomeric forms of either CTLA4 or CD28". In an attempt to overcome this problem, fusion constructs were generated by attaching Ig constant domains to the monomeric forms of CTLA4 and CD28. Peach *et al.* refers loosely throughout the paper to the CTLA-4 and CD28 fusion proteins as "soluble". In fact, however, these fusion constructs are not truly soluble. This is made clear on page 2057, where it is stated:

> "We previously reported that CTLA4Ig and CD28Ig form higher molecular weight aggregates in solution... . Preliminary experiments with size exclusion chromatography showed that homologue scan mutants also existed as oligomers, but that the degree of oligomerization did not correlate with binding activity. ... [T]he specific activity of B7-1 binding was constant for different size oligomers of fusion protein HS7...".

Therefore, the constructs described in Peach *et al.* are not soluble in the sense of the word as defined in the instant application, *i.e.* existing as discrete, non-aggregated molecules in aqueous solution, in the absence of detergents or other solubilizing entities. In fact, not only were both the original chimeric proteins and the mutant chimeric proteins of Peach *et al.* in the form of aggregates, but each preparation existed in a range of aggregate sizes which could be separated by size exclusion chromatography.

In summary, it is not possible to draw any valid conclusions regarding relative solubilities of the mutant chimeric proteins generated by Peach *et al.* Figure 4 provides no measure solubility (in view of the presence of SDS), and the brief discussion of solubility of the constructs on page 2057 simply indicates that the mutants were not soluble (they existed as oligomers). Therefore, the claims are not anticipated by Peach *et al.*, as the teachings of Peach *et al.* do not result in improved solubility, as is required by claim 1.

Claims 1-9, 13, 15, 16, 18, 19, 21, 28 and 33 were rejected under 35 U.S.C. §102(e) as allegedly being anticipated by Koide *et al.* The rejection is traversed.

Koide relates to binding polypeptides which use fibronectin type III (Fn3) as a scaffold. On page 10, the Office Action alleges that Fn3 is a monomeric V-like domain that has "BC", "DE" and "FG" loops that correspond to CDRs 1, 2 and 3 of antibodies. In support of this assertion, the Examiner refers to paragraph 97 of Koide which states:

"The structure is best described as a β sandwich similar to that of AB VH domain except that Fn3 has seven β-strands instead of nine (Fig. 1). There are three loops on each end of Fn3; the positions of the BC, DE and FG loops approximately correspond to those of CDR1, 2 and 3 of the VH domain, respectively (Fig. 1 C,D)."

Although this paragraph may appear to disclosure similarities between the domain of Fn3 and V-like domains, in fact it highlights important differences, which a skilled person would understand and appreciate. For example, this paragraph points out that Fn3 has seven β-strands, instead of nine. A skilled person would understand that this means that Fn3 does not possess a V-like domain, rather it possesses an S-like domain. Again, we refer to Bork *et al.* (Appendix G). As is clear from Figure 1 on page 311, proteins falling within the definition of a v-type IgSF member have a different topology from s-type IgSF proteins. V-set IgSF proteins have 9 β strands, whereas s-type IgSF proteins have 7 β strands. Table 1 on page 310 includes Fn3 in the so-called "s-type" category of structures, which are distinct from the "v-type" structures. Among those proteins described as having a v-type topology are CD2, CD4 and CD8.

Indeed, Fn3 is a whole IG superfamily within its own right, as is well-known within the art. For example, paragraph 96 of Koide states:

"Fibronectin is a large protein which plays essential roles in the formation of extracellular matrix and cell-cell interactions; it consists of many repeats of three types (I, II and III) of small domains (Baron et al., 1991). Fn3 itself is the paradigm of a large subfamily (**Fn3 family or s-type Ig family**) of the immunoglobulin superfamily (IgSF). The Fn3 family includes cell adhesion molecules, cell surface hormone and cytokine receptors, chaperonins and carbohydrate-binding domains..." (emphasis added).

Further evidence of Applicants' position is found in Barclay *et al.* (Appendix I), where, in the penultimate paragraph on page 38, the FN type III superfamily is clearly differentiated form the IG superfamily. Page 42 also contains a description of the FN type III superfamily, and refers to Figure 4 on page 51. It is evident from Figure 4 that an FN type III domain only contains seven β strands, and not nine, as are found in V-like domains. In addition, on page 54, in the section discussing the FN type III superfamily, it states:

> "Structures of FN type IIISF domains have recently been solved by NMR and X-ray crystallography. This domain consists of two β sheets with a similar folding pattern tot he IgSF fold, the CytokineR domain and the domains of the PapD chaperone protein.

However, there is no significant sequence similarity amongst these proteins as analyzed by the methods discussed above."

The disclosures on pages 44 and 46 of Barclay *et al.* make it clear that a combination of structural similarity and sequence similarity as defined through computer analysis is necessary to define a domain type.In view of the above, it is clear that Fn3 does not comprise a V-like domain, but instead comprises an S-like domain. Furthermore, there is nothing in Koide to suggest that modifications described in relation to Fn3 could be applied to monomers comprising V-like domains. Consequently, Koide cannot anticipate the instant claims.

Claims 1-21, 28 and 33 were rejected under 35 U.S.C. §102(f) because the applicant allegedly did not invent the claimed subject matter. The rejection is traversed.

The inventors of the present application are Gregory Coia, Maria Galanis, Peter Hudson, Robert Irving and Stewart Nuttall. The authors of Nuttall *et al.*, 1999 are Stewart Nuttall, Mat Rousch, Robert Irving, Simon Hufton, Hennie Hoogenboom and Peter Hudson. The Office Action requests that the contributions of each inventor be clarified in view of the Nuttall *et al.* reference.

Drs. Hudson and Irving are responsible for the initial broad concept of the invention, so their contribution was to all claims. Dr. Coia was responsible for the claims related to the ribosomal display library (claims 30-32). This work was not reported in Nuttall *et al.* If the current restriction requirement is maintained and claim 30-32 are cancelled, Dr. Coia will be removed as an inventor. Dr. Galanis contributed at least to claims 1, 36, 38 and 40 (and dependent claims), as she discovered the improved solubility of modified CTLA-4 using HPLC. This work was not specifically discussed in Nuttall *et al.* Dr. Nuttall performed the CDR substitutions, which contributed to at least claims 1, 3, 36, 38, 40 (and dependent claims).

It will be appreciated by the Examiner that, in the scientific community, persons are or are not included as authors on scientific articles for reasons that do not involve issues of inventorship. Any work reported in Nuttall *et al.*, particularly work that may be common to the present application, was performed by or under the direction, supervision or control of the inventors on the present application.

In view of the foregoing, reconsideration and withdrawal of the rejections under 35 U.S.C. §102 are requested.

Restriction Requirement

Applicants maintain that unity of invention does exist, and that the claims define a contribution over the prior art. As discussed above, the claims are novel over Peach *et al.* As such, it is requested that the requirement for restriction be reconsidered, and that the claims of group IV be searched and examined with the claims of group I.

Claims 22-27 of group IV are drawn to a polynucleotide encoding a modified monomeric VLD binding moiety, vectors and host cells comprising, and methods of producing the modified monomeric VLD binding moiety by culturing said host cells, wherein the monomeric V-like domain is derived from a non-antibody ligand that is CTLA-4. Claims 1-11, 13-21, 28 and 33 of group I are drawn to a modified monomeric VLD binding moiety wherein the VLD is derived from a non-antibody ligand that is CTLA-4. According to the PCT practice guidelines, a group of inventions is considered to form a single inventive concept where there is a technical relationship among the inventions that involves at least one common or corresponding technical feature. The present case represents the very example of this in that the Group I and Group IV claims share a special technical feature. Specifically, the Group IV claims are directed to polynucleotides encoding a modified monomeric VLD binding moiety according to claim 1 or 21 (claim 22), a vector comprising said polynucleotide (claim 23), a host cell transformed with said vector (claims 24-25) and a method of producing a modified monomeric binding moiety in which said host cells are cultured (claims 26-27).

Annex B to the Administrative Instructions under the PCT, Part 2, "Examples Concerning Unity of Invention", further provides appropriate guidance for the present case. Specifically, Example 17 relates to a claim 1 drawn to a "Protein X" and a claim 2 drawn to a "DNA sequence encoding protein X". The guidelines clearly state that if "[e]xpression of the DNA sequence in a host results in the production of a protein which is determined by the DNA sequence", and if "[t]he protein and the DNA sequence exhibit corresponding special technical features", then there is unity between claim 1 and claim 2. This example is directly applicable to claims 1, 21 and 22 of the present case. A copy of Annex B is attached as Appendix K, for the Examiner's convenience.

In addition, the method claims of group VII (claim 29) and group X (claims 30-32) share the special technical feature of at least one modified monomeric VLD, derived from a non-antibody ligand, with improved solubility over an unmodified VLD, wherein the modified VLD

functions as a binding moiety. This special technical feature is a novel contribution over the prior art, and therefore, unity of invention exists among the claims.

In view of the above arguments, distinguishing the instant invention from Peach *et al.*, it is submitted that the modified VLD binding moiety of claim 1, comprising a monomeric VLD having at least one CDR loop structure modified or replaced to improve solubility of the VLD, is the special technical feature that links groups I, IV, VII and X to form a single inventive concept. Thus, Example 17 applies, unity of invention exists, and the claims of groups I, IV, VII and X (and groups II, III, V, VI, VIII and IX, if the linking claims are found allowable) should be examined in a single application. Therefore, reconsideration and redrawing of the restriction requirement are requested.

V. THE REJECTIONS UNDER 35 U.S.C. §103 ARE OVERCOME

Claims 1, 13 and 14 are rejected under 35 U.S.C. §103(a) as allegedly being unpatentable over Koide in view of Bogden *et al.* Claims 1 and 15-17 are rejected under 35 U.S.C. §103(a) as allegedly being unpatentable over Koide in view of Cai *et al.* The rejections are traversed, and will be addressed collectively.

Initially, it should be noted that the skilled person, on looking to improve the solubility of a binding moiety comprising a monomeric V-like domain, without adversely affecting the binding properties, would not have considered modifying the CDR regions for two reasons. First, the CDR regions are integral to the binding properties. Second, the prior art teaches directly away from such an action. Indeed, the prior art is replete with examples in which the VLDs of binding moieties are combined with solubilizing factors. For example, as discussed above Peach *et al.* involves fusing CTLA-4 with an Ig fragment to improve solubility. Nowhere in the prior art is modifying the CDR(s) within VLDs, in order to improve the solubility of binding moieties, reported or discussed. Consequently, there was no motivation for the skilled person to even consider modifying the CDR(s) within the VLD in order to improve solubility. Furthermore, even if the skilled person had considered modifying the CDR(s), and there is no evidence to indicate that this is the case, s/he would not have done so with a reasonable expectation of success.

As was discussed with respect to the anticipation rejection, Koide relates to Fn3, which is does not comprise a VLD. Bogden *et al.* relates only to somatostatin as an inhibitor of tumor growth, but does not teach or suggest placing somatostatin or any part thereof, in the CDR loop

structure of a VLD. Since neither Koide nor Bogden *et al.* involve a molecule comprising a monomeric VLD, an essential element of the claims is missing in the combination of these two references. The same is true for Cai *et al.*, which relates only to the human anti-melanoma antibody, V86. Cai *et al.* does not teach or suggest a molecule comprising a monomeric VLD, neither does Koide, and therefore, the claims cannot be obvious over any of these references in any combination.

Accordingly, reconsideration and withdrawal of the rejections under 35 U.S.C. §103 are requested.

CONCLUSION

As it is believed that this application is in condition for allowance an early notice to that effect is earnestly solicited. If, however, there remains any issue outstanding, the Examiner is invited to contact the undersigned for its prompt attention.

Respectfully submitted,

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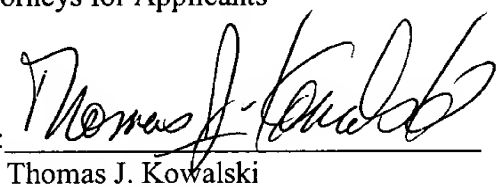
By: 

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